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Longitudinal Loss of Gray Matter Volume in Patients with First-Episode Schizophrenia: DARTEL Automated Analysis and ROI Validation

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Abstract

Region of Interest (ROI) longitudinal studies have detected progressive gray matter (GM) volume reductions in patients with first-episode schizophrenia (FESZ). However, there are only a few longitudinal voxel-based morphometry (VBM) studies, and these have been limited in ability to detect relationships between volume loss and symptoms, perhaps because of methodologic issues. Nor have previous studies compared and validated VBM results with manual Region of Interest (ROI) analysis.

In the present VBM study, high-dimensional warping and individualized baseline-rescan templates were used to evaluate longitudinal volume changes within subjects and compared with longitudinal manual ROI analysis on the same subjects. VBM evaluated thirty-three FESZ and thirty-six matched healthy control subjects (HC) at baseline (cross-sectionally) and longitudinally evaluated 21 FESZ and 23 HC after an average of 1.5 years from baseline scans. Correlation analyses detected the relationship between changes in regional GM volumes in FESZ and clinical symptoms derived from the Brief Psychiatric Rating Scale, as well as cognitive function as assessed by the Mini-Mental State Examination.

At baseline, patients with FESZ had significantly smaller GM volume compared to HC in some regions including the left superior temporal gyrus (STG). On rescan after 1.5 years, patients showed significant GM volume reductions compared with HC in the left STG including Heschl's gyrus, and in widespread brain neocortical regions of frontal, parietal, and limbic regions

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including the cingulate gyrus. FESZ showed an association of positive symptoms and volume loss in temporal (especially STG) and frontal regions, and negative symptoms and volume loss in STG and frontal regions. Worse cognitive function was linked to widespread volume reduction, in frontal, temporal and parietal regions. The validation VBM analyses showed results similar to our previous ROI findings for STG and Cingulate Gyrus. We conclude FESZ show widespread, progressive GM volume reductions in many brain regions. Importantly, these reductions are directly associated with a worse clinical course. Congruence with ROI analyses suggests the promise of this longitudinal VBM methodology.

Keywords

first episode; longitudinal study; VBM; positive symptom; negative symptom; cognitive function

1. Introduction

Numerous cross-sectional magnetic resonance imaging (MRI) studies indicate smaller gray matter (GM) volume in schizophrenia patients at first episode (FESZ) compared with healthy controls (HC) (reviewed in (Glahn et al., 2008; McCarley et al., 1999b; Shenton et al., 2001)). The initially controversial hypothesis of post-onset progressive GM loss in FESZ has gained support through many recent studies (see, for example, citations (Bachmann et al., 2004; Cahn et al., 2002; Hoff et al., 1999; Mane et al., 2009; van Haren et al., 2008; Whitford et al., 2006) and below). One method used in many studies demonstrating longitudinal change is manually drawn Region of Interest (ROI) analysis. A previous longitudinal analysis of neocortical gray matter (NCGM) volume changes from our laboratory (Nakamura et al., 2007) showed loss of overall NCGM volume over 1.5 years, with a higher rate in temporal and frontal lobes. Other longitudinal ROI studies from our laboratory have shown progressive GM volume reduction in cingulate gyrus (CG) (Koo et al., 2008) and in superior temporal gyrus (STG) and STG components of Heschl's Gyrus (HG) and planum temporal (Kasai et al., 2003a; Kasai et al., 2003b); another group has recently confirmed progression in STG and its components (Takahashi et al., 2009b). Of note, we found the degree of longitudinal volume reduction using ROI methods was greater in certain gyri (e.g., STG, CG) compared with overall NCGM, suggesting regional differences in progression.

A second method is voxel-based morphometry (VBM), defined by Ashburner and Friston (Ashburner and Friston, 2000) as "a voxel-wise comparison of the local concentration of GM between two groups of subjects". To detect regional group differences, VBM has the advantages over manual ROI methods of allowing whole brain coverage and less laborious processing. However, there is a lack of uniformity in results in VBM studies. One reason is differences in methods among the previous longitudinal analyses, and those results were not compared with results of the manual ROI analyses using the same subjects. In addition, many of the previous longitudinal VBM studies have not reported structural-symptomatic associations although they have demonstrated GM volume loss in several brain regions in FESZ (Farrow et al., 2005; Mane et al., 2009; Theberge et al., 2007; Whitford et al., 2006).

In the current study, we conducted whole brain VBM analysis to investigate progressive GM volume changes in FESZ compared with HC. For this analysis, we developed a new longitudinal VBM method. The DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra) (Ashburner, 2007) tool in the Statistical Parametric Mapping (SPM) 5 was used to evaluate within-subject changes by creating individual templates. To establish our new method (Giuliani et al., 2005), validation VBM with small volume correction (SVC) analyses were conducted for STG, HG, and CG using the same subjects

and scans of our previous manual ROI analyses (Kasai et al., 2003a; Kasai et al., 2003b; Koo et al., 2008), and results were compared. Finally, exploratory correlation analyses were conducted between changes in regional GM volumes and cognitive function and positive and negative symptoms to understand the pathology of these symptoms in FESZ.

2. Material and Methods

2.1. Subjects

Thirty-three FESZ and 36 HC were compared in a cross-sectional study (**Table 1**). The patients were recruited from inpatient units at McLean Hospital, Belmont, Massachusetts. HC were recruited from the local community through newspaper advertisements. Consistent with our previous studies (Salisbury et al., 2007; Salisbury et al., 1998), “first episode” was operationally defined as the first hospitalization for psychosis (all except 6 subjects in the present cross-sectional study) or within one year of the first hospitalization for psychosis. Inclusion criteria for patients and HC were age 18 to 45, IQ above 75, and no history of seizures, head trauma with loss of consciousness, neurologic disorder, or an alcohol or drug detoxification within the last 5 years. Patient diagnosis was based on the Structured Clinical Interview for *DSM (SCID) Patient Edition for DSM-III-R* (Spitzer et al., 1990c) or *DSM-IV* (First et al., 1997) criteria. The HC were confirmed to have no Axis I or II disorders using *SCID-Non-Patient Edition* (Spitzer et al., 1990a) and *SCID-II* interviews (Spitzer et al., 1990b), and no history of Axis I disorders in their first-degree relatives per self-report.

Twenty-one FESZ and 23 HC were rescanned approximately 1.5 years later (**Table 2**). The groups in the cross-sectional and longitudinal studies were matched for age, gender, parental socioeconomic status (PSES) (Hollingshead, 1965), and handedness (Oldfield, 1971). Exclusion of the single mildly left-handed FESZ patient (Edinburgh index = -0.07) from the subsequent analyses did not change the results of the cross-sectional or longitudinal study.

Medication history before and during the first hospitalization and between scans, if present, was assessed from patient reports and hospital records. The median duration of antipsychotic medication (typical or atypical antipsychotics) before baseline scan was 1 week (range 0-20 weeks: **Table 1**). In this naturalistic sample, 4 FESZ reported medication noncompliance for more than 3 months before the follow-up scan and 1 FESZ received no medication at the follow-up scan. 15 FESZ received atypical antipsychotics and 1 FESZ received both typical and atypical antipsychotics (**Table 2**). Daily chlorpromazine equivalent antipsychotic dosage (Woods, 2003) did not correlate with any volumes or volumetric changes (detailed information on antipsychotics and mood stabilizer are included in **Table 1 and 2**).

The Mini-Mental State Examination (MMSE) (Folstein et al., 1975) and the Wechsler Adult Intelligence Scale-Revised or III (WAIS-R, WAIS III) (Wechsler, 1981, 1997) were performed on all subjects. The MMSE was used to rule out any dementia or delirium and to evaluate cognitive function. The information subscale of the WAIS was used to estimate general fund of information, and digit span subscales were used to test immediate and short-term memory, attention, and concentration. The patients' severity of illness and general level of functioning were evaluated with the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) and the Global Assessment Scale (GAS) (Endicott et al., 1976). This study was approved by the McLean Hospital, Veterans Affairs Boston Healthcare System, and Harvard Medical School Institutional Review Boards. All subjects gave written informed consent before participation.

2.2. MRI processing

The MRI protocol used two pulse sequences on a 1.5-T MRI system (GE Medical System, Milwaukee, Wisconsin), as described (Hirayasu et al., 2000). Briefly, a three-dimensional

Fourier transformed spoiled-gradient-recalled (SPGR) acquisition sequence yielded a coronal series of contiguous 1.5-mm images (echo time 5msec, repetition time 35msec, repetition 1, nutation angle 45°, field of view 24cm, acquisition matrix 256 × 256 × 124, voxel dimension 0.9375 × 0.9375 × 1.5 mm). The same scanner was used for both the baseline and follow-up scans.

2.3. Image preprocessing: Baseline cross-sectional study

The theory and algorithms of VBM using the SPM5 software (Wellcome Department of Cognitive Neurology, London, UK) have been well documented (Ashburner and Friston, 2000, 2005). A detailed protocol for the cross-sectional analysis is provided in Supplemental Materials. Briefly, following realignment, T1-weighted images were segmented into GM, white matter (WM), and cerebrospinal fluid (CSF) probability maps. All the GM images were spatially non-linearly normalized to the *population* template which was created by GM and WM maps and DARTEL, and then, Jacobian modulated. These images were affine transformed to MNI (Montreal Neurological Institute) space, and finally, were smoothed with an 8-mm full-width at half-maximum (FWHM) Gaussian kernel.

2.4. Image preprocessing: longitudinal study

As described in the flow chart of **Figure 1**, SPM5 was also used to evaluate group effects of longitudinal volume changes based on individual volume changes. A key element of the pipeline was the use of an individualized baseline-to-follow-up DARTEL-based template in order to provide more precise spatial alignment.

2.5. Statistical analysis: cross-sectional study

The framework of the general linear model was employed for estimating group differences in regional GM volumes at baseline scan using a two sample *t* test. The resulting set of voxel values for each contrast constituted a statistical parametric map of the *t* statistic [SPM(*t*)]. In the text and tables, we discuss only those results that survived a correction at .05 (False Discovery Rate (FDR)-corrected) (Genovese et al., 2002) for the volumes searched.

2.6. Statistical analysis: longitudinal study

A whole brain longitudinal VBM analysis was conducted as follows. First, the smoothed images, which were generated from step 5 of image preprocessing, were entered into a design matrix in four conditions: FESZ baseline scan images, FESZ follow-up scan images, HC baseline scan images, and HC follow-up scan images. Forty-four subject-specific, dummy covariates (i.e., 21 FESZ and 23 HC) modeled the variance attributable to repeated measures within each subject (Whitford et al., 2006). Voxel-wise paired *t* tests were used to investigate for progressive GM changes within the FESZ and HC groups. An exclusion-mask technique, as discussed by Job and colleagues (2005) (Job et al., 2005), was used to explore the differential patterns of progressive GM change exhibited by the FESZ and HC. This technique uses masking to exclude any voxel level changes over time in the HC (paired *t* test, *p*<.05 uncorrected) from the changes in FESZ (paired *t* test, *p*<.001 uncorrected). Groups were not directly compared in this analysis, however, any significant changes in the HC are excluded leaving only changes to the FESZ. This statistical technique has been used in several previous VBM and functional neuroimaging studies (Cabeza et al., 2004; Critchley et al., 2000; Job et al., 2005; Morcom et al., 2003). The SPM(*t*) maps in Figure 3 are displayed at an uncorrected threshold of *p*<.001 with an extent threshold of 70 voxels for graphical reporting only. In the text and tables, we discuss only those results that survived a correction at FDR-corrected *p*<.05 for the volumes searched.

To validate our longitudinal VBM methodology and to facilitate comparisons, we also performed SVC analyses for: a) the current participant sample; and b) the same FESZ and HC subjects that we investigated in our previous studies in which we used manually-drawn ROIs (Kasai et al., 2003a; Kasai et al., 2003b; Koo et al., 2008). Based on the results of these studies, SVC analyses were conducted for the 12 ROIs including bilateral STG, amygdala, and hippocampus (based on the results of Kasai and colleagues (2003) (Kasai et al., 2003a), bilateral HG (based on the results of Kasai and colleagues (2003) (Kasai et al., 2003b), and bilateral anterior and posterior CG (A/PCG, based on the results of Koo and colleagues (2008) (Koo et al., 2008)). These ROIs were derived from the WFU-Pickatlas (Maldjian et al., 2003) and were created based on the Talairach Demon in the MNI space (Lancaster et al., 1997; Lancaster et al., 2000; Talairach and Tournoux, 1988) (Figure S1 in Supplemental Materials). Significance thresholds were set at FDR- $p < .05$ with an extent threshold of 80 voxels.

2.7. Volume-Symptom correlations

Given the ordinal nature of the clinical rating scales, Spearman's correlations were used to investigate the association between patients' degree of change in the ROI-defined volumes (calculated as per the procedure of Whitford and colleagues (2005) (Whitford et al., 2005) – see supplemental material) and their change in score on the clinical variables, namely the BPRS thinking-disturbance factor (positive symptom) score and its components (hallucinatory behavior, unusual thought contents, and conceptual disorganization), the BPRS withdrawal-retardation factor (negative symptom) score (Overall and Gorham, 1962; Overall and Klett, 1972), and MMSE total score. Although multiple correlations were performed, results are reported as $p < .05$ (two-tailed); hence caution in interpretation is needed due to the exploratory nature of the evaluation.

3. Results

There were no significant group differences in age, gender, handedness, or PSES. The patients had lower socioeconomic status, less education, lower MMSE at second scan, and lower WAIS-R performance, consistent with reduced functioning due to the disorder. Interscan interval time did not differ between the two groups (**Table 1** and **2**).

3.1. Baseline cross-sectional study

The result of the cross-sectional study showed that the 33 FESZ had significantly smaller GM volumes in the left STG, insula, amygdala, hippocampus, bilateral thalamus, right ACG (rostral subregion) and superior frontal gyrus compared with 36 matched HC at baseline (FDR-corrected $p < .05$, **Figure 2**, Supplemental Table S1, and Figure S2). We found no region where the FESZ had a significantly larger volume compared with the HC.

3.2. Longitudinal study

The paired t test (i.e., baseline vs. follow-up scan) within the 21 FESZ showed widespread significant progressive GM volume reductions, especially in the frontal and temporal lobes, but did not show any progressive GM volume increases. The paired t test within the 23 HC showed no progressive GM volume changes. The group comparison using the excluding mask function, which allowed a measure of progressive GM volume reduction in the FESZ while excluding the volumetric changes associated with normal aging, also showed significantly reduced GM volume in extensive brain regions. These regions included the temporal regions (bilateral STG including HG), frontal regions (bilateral superior, middle, and inferior frontal gyrus (S, M, IFG), orbitofrontal cortex, and precentral gyrus), parietal regions (bilateral postcentral gyrus and supramarginal gyrus, and right angular gyrus and superior parietal gyrus), limbic regions (bilateral insula, ACG (bilateral rostral and dorsal

subregions, and left subgenual subregion), and PCG, and right cerebellum (**Table 3** and **Figure 3**). An alpha of $p < .05$ FDR-corrected was used throughout.

In addition, we also conducted ROI comparisons in the bilateral STG, HG, amygdala, and hippocampus, as well as ACG and PCG between the 21 FESZ and 23 HC ($p < .05$ FDR-corrected for small volumes). The FESZ were observed to lose a significantly greater volume of GM compared to the HC over the follow-up interval in the left STG, left HG, bilateral ACG (rostral, dorsal, and subgenual subregion) and PCG (Supplemental materials Figure S3).

Finally, to confirm our previous ROI results using this new longitudinal VBM method, we conducted three VBM analyses ($p < .05$ FDR-corrected for small volumes). Each validation study used the same subjects as its equivalent ROI study (hence with different subject numbers in the validations). In the first SVC analysis for bilateral STG, amygdala, and hippocampus (corresponding to Kasai et al., 2003a), progressive GM volume reduction was shown only in the left STG. No progressive GM reductions were observed in the right STG, bilateral amygdala or hippocampus in the 14 FESZ compared with 13 HC. The second SVC analysis for bilateral HG (corresponding to Kasai et al., 2003b) showed GM volume reduction only in the left HG in the 14 FESZ compared with 22 HC. In the last validation analysis for bilateral ACG and PCG (corresponding to Koo et al., 2008), progressive GM volume reduction was observed in the bilateral ACG (rostral, dorsal, and subgenual subregions) and PCG in the 17 FESZ compared with 18 HC (Supplemental materials Figures S4).

3.4. Correlation analysis

Correlation analyses showed significant relationships between the degree of progressive GM volume loss in certain brain regions and longitudinal changes in clinical scores in the 21 FESZ. Approximately half of the patients improved in their clinical status, as shown by lower symptom ratings at retest. Patients' longitudinal change in positive symptoms (BPRS thinking disturbance factor), negative symptoms (BPRS withdrawal-retardation factor) and cognitive performance (MMSE) correlated with their degree of longitudinal GM change.

Positive symptoms—Less improvement in the BPRS thinking disturbance factor was correlated with more longitudinal GM volume loss of HG (bilateral: $\rho = -0.62$, $p = .003$, left: $\rho = -0.55$, $p = .010$, right: $\rho = -0.67$, $p = .001$). A lesser improvement in hallucinatory behavior was associated with more GM volume loss of HG (bilateral: $\rho = -0.67$, $p = .001$, left: $\rho = -0.63$, $p = .002$), while less improvement in unusual thought content was correlated with more GM volume loss of right HG ($\rho = -0.55$, $p = .010$) (**Figure 4** and Supplemental Figure S5). In addition, diminished improvement in thinking disturbance was correlated with more volume loss of the right precentral gyrus ($\rho = -0.46$, $p = .035$), left insula ($\rho = -0.47$, $p = .033$) and the right postcentral gyrus ($\rho = -0.46$, $p = .038$).

Negative symptoms—Less improvement on the BPRS withdrawal-retardation factor was correlated with more longitudinal GM volume loss in: frontal lobe regions (IFG; left: $\rho = -0.45$, $p = .040$, right: $\rho = -0.54$, $p = .011$, left SFG: $\rho = -0.46$, $p = .038$); fronto-limbic regions (bilateral insula; $\rho = -0.55$, $p = .010$, right ACG: $\rho = -0.44$, $p = .047$); temporal lobe regions (STG; left: $\rho = -0.44$, $p = .046$, bilateral: $\rho = -0.47$, $p = .033$); and in the parietal region of left supramarginal gyrus ($\rho = -0.52$, $p = .016$) (**Figure 5** and Supplemental Figure S6).

Cognitive function—MMSE scores showed that approximately half of the 21 FESZ improved, while the others got worse. Overall, less improvement on MMSE correlated with

a larger longitudinal GM volume loss in: frontal lobe regions (MFG; bilateral: $\rho=0.49$, $p=.026$, left: $\rho=0.45$, $p=.041$, right: $\rho=0.46$, $p=.036$, IFG; bilateral: $\rho=0.51$, $p=.019$, left: $\rho=0.50$, $p=.022$, right: $\rho=0.50$, $p=.021$, precentral gyrus; left: $\rho=0.48$, $p=.029$, right: $\rho=0.61$, $p=.003$), fronto-limbic regions (left insula: $\rho=0.49$, $p=.024$, left PCG: $\rho=0.49$, $p=.026$); the temporal region of left STG ($\rho=0.44$, $p=.048$); and the parietal region of right postcentral gyrus ($\rho=0.54$, $p=.012$) (Supplemental Figure S7). No particular MMSE item accounted for the decreased improvement/worse score associated with these regional changes. Further indicating a lack of specificity was a significant correlation between MMSE and overall neocortical volume ($\rho=-0.48$, $P=0.29$). In contrast, overall neocortical volume was not significantly correlated with either positive or negative symptom BPRS factors.

In partial correlation analyses controlling for the overall neocortical volume, the MMSE and negative symptom factor showed no associations with any regional GM volume changes, while the positive symptom factor showed significant relationships with volume loss of HG (bilateral: $\rho=-0.57$, $p=.009$, left: $\rho=-0.46$, $p=.042$, right: $\rho=-0.64$, $p=.003$).

4. Discussion

This study found that FESZ exhibited GM abnormalities at baseline and showed widespread progressive GM reduction in frontal, temporal and parietal lobes over the first 1.5 years of illness. Distinctive features of this study were: 1) the congruence of longitudinal results using DARTEL-based VBM and ROI methodology; and 2) the correlation of patients' levels of longitudinal GM reduction with the longitudinal changes in their clinical symptoms and basic cognitive functioning. We believe the richness of FESZ GM loss-symptom associations detected in this study is unique in the longitudinal literature, even though they were obtained by relatively liberal, exploratory analyses. This study thus emphasizes the clinical relevance of progression of gray matter loss in the period immediately following onset of schizophrenia.

First, the whole brain longitudinal analysis demonstrated GM volume loss in widespread brain regions in temporal, frontal, fronto-limbic, and parietal regions in the 21 FESZ compared with 23 HC.

In the temporal lobe, progressive GM volume reductions were present in bilateral STG and HG. Medial temporal lobe showed smaller left amygdala-hippocampal complex GM volume at first hospitalization, a variable on which the literature shows inconsistent findings (Glahn et al., 2008; Honea et al., 2005; Shenton et al., 2001; Velakoulis et al., 1999). The present study found no medial temporal lobe volume losses longitudinally, consistent with a previous longitudinal VBM analysis (Whitford et al., 2006) and a recent meta-analysis (Olabi et al., 2011).

In the frontal lobe, the longitudinal analysis uncovered widespread frontal GM reduction, consistent with our previous ROI analysis (Nakamura et al., 2007) and other VBM longitudinal studies of FESZ (Theberge et al., 2007; Whitford et al., 2006). Progressive GM loss was found in all surface frontal gyri: superior, middle, inferior, precentral, and orbitofrontal. These findings are more extensive than the specific IFG loss previously reported (Whitford et al., 2006), possibly due to improved sensitivity of the DARTEL methodology (Tahmasebi et al., 2009), although other reasons like subject differences cannot be ruled out.

Within fronto-limbic regions, bilateral rostral and dorsal subregions and the left subgenual subregion of the ACG, and left PCG showed progressive volume reductions. These findings were consistent with our previous manual ROI analysis (Koo et al., 2008).

Within the parietal lobe, the longitudinal analysis detected extensive progressive GM volume reductions in FESZ in bilateral postcentral and supramarginal gyri, and right angular and superior parietal gyri. The lateral parietal cortex, medial prefrontal cortex, ventral ACG and PCG are thought to be a part of the so-called default network (Raichle et al., 2001; Whitfield-Gabrieli et al., 2009), in which some functional MRI studies have reported hyperactivity in SZ (Garrity et al., 2007; Whitfield-Gabrieli et al., 2009). The structural abnormalities in frontal, frontal-limbic, and parietal regions detected in this study might contribute to functional abnormalities in the default network.

Second, the SVC analyses using both the current subjects and the same subjects as in our previous ROI analyses demonstrated similar results as the ROI analyses (Kasai et al., 2003a, 2003b; Koo et al., 2008), thus supporting our new technology. Although it remains for us to confirm the congruence of VBM-ROI results in other brain regions, such as frontal and parietal regions, the present similarity of our VBM results with previous ROI analysis suggest the validity and reliability of our new method. As a final point, we mention that, to the best of our knowledge, there are no longitudinal VBM studies comparing their results with manual ROI results using the same subjects, although such comparisons would, in our opinion, be useful.

Finally, correlational analyses demonstrated many associations between the degree of GM reduction and clinical symptoms. While many associations indicated GM loss in temporal regions were related to positive symptoms and volume loss in frontal regions were related to negative symptoms, the data showed a more complex interplay between brain regions with volume reduction in the production of symptoms. Abnormalities were found in the STG/HG and postcentral gyrus, which have both early stage auditory and somatosensory processing functions and also in regions with more complex processing and top down control functions (frontal gyri). This is in accord with a conceptualization of schizophrenia as involving disturbances in both bottom up and top down stages of processing. Functionally the temporal lobe STG regions are substrates of auditory and language processing (Galaburda et al., 1978), two domains which are often impaired in SZ. Several previous functional and structural neuroimaging studies indicated that cross-sectional temporal lobe abnormalities were related to positive symptoms (Bachmann et al., 2004; Dierks et al., 1999; Shenton et al., 1992; Wible et al., 2001b). Our data suggest that longitudinal temporal lobe GM loss is linked to worsening or less improvement in positive symptoms over time. Our previous ROI study showed HG volume reduction was associated with reduction in the auditory mismatch negativity to pitch (Salisbury et al., 2007). Thus worsening in both positive symptoms and auditory pathophysiology appear to be associated with progressive temporal lobe GM reduction. Importantly, there was also support for the concept of a complex interplay of brain regions in positive symptom production, with both insula and pre- and post-central gyri showing positive symptom associations.

Frontal regions play important roles in the executive functions of attention, working memory, set shifting, and planning, as well as in learning and memory and regulation of emotion and social interaction (Gazzaniga, 2005). Deficits in these functions may appear in schizophrenia as negative symptoms and cognitive dysfunction (Hirsch and Weinberger, 2003). Several neuroimaging studies have, in fact, reported a relationship between cross-sectional frontal volume reductions and negative symptomatology and cognitive dysfunction (Mathalon and Ford, 2008; Weinberger et al., 1986; Wible et al., 2001a; Wolkin et al., 1992; Zipparo et al., 2008). In terms of longitudinal studies, some previous studies have reported such relationships in FESZ. In the previous ROI studies, prefrontal GM declines have been reported to be associated with greater BPRS negative symptom scores (Mathalon et al., 2001). With respect to insula, an ROI study (Takahashi et al., 2009a) has reported associations of insular cortex progression and BPRS negative symptoms, similar to that

reported here using VBM methodology. An association of right ACG volume reduction with the BPRS withdrawal-retardation factor (negative symptoms) was also found in our ROI study (Koo et al., 2008), further suggesting the validity of our VBM approach. Thus, our results of the associations between negative symptoms and frontal and fronto-limbic progressive GM reductions were consistent with these previous findings. Reinforcing the notion of a complex regional interplay in symptom production was the STG GM volume reduction association with negative symptoms.

General cognitive changes as indexed by the MMSE were associated with widespread GM loss, although frontal associations predominated. The lack of domain-specific MMSE cognitive correlations suggests the MMSE correlations index a diffuse cognitive impairment that occurs over time in FESZ. We note that the MMSE has been validated in cognitive decline (Cockrell and Folstein, 1988), but not in schizophrenia. Validation of its use in schizophrenia will thus require comparison with other more standard cognitive tests. Our data showing the strong MRI volumetric correlations suggest this comparison for validation might be worthwhile.

The precise neurobiological mechanism that underlies this progressive, perhaps neurodegenerative, change shown in the current longitudinal VBM analysis is unknown. However, recent studies have provided some evidence that neuropil reduction and not cellular loss has been shown to be the basis of GM loss in both temporal (Sweet et al., 2003) and frontal regions (Selemon and Goldman-Rakic, 1999) in patients with SZ. Such loss likely underlies the GM loss observed here. While the neurobiological mechanism underlying GM volume/neuropil loss is unknown, we have speculated that a cortical circuit abnormality (deficient recurrent inhibition as a result of γ -aminobutyric acid [GABA]-ergic abnormalities in parvalbumin-positive interneurons) and consequent excitotoxic reduction in neuropil might be responsible (McCarley et al., 1996; McCarley et al., 1999a). This model is compatible with glutamatergic hypofunction of pyramidal neurons' recurrent collaterals on the *N*-methyl-D-aspartate (NMDA) glutamate receptor on GABAergic interneurons (Coyle, 1996), and is now recognized as a plausible mechanism (see review (Krystal et al., 2003; Woo et al., 2010)). Relevant to our model, a MRS study (Theberge et al., 2007) found progressive glutamatergic abnormalities that were compatible with excitotoxicity, although also compatible with neuroplastic changes.

Limitations. First, the correlation analysis of this study must be labeled as exploratory and therefore needing confirmation in future planned studies because of the number of correlations calculated. However, taken as a whole, our GM volume and clinical correlation data do show statistically significant associations. We used 3 major clinical variables, 2 BPRS factors (positive and negative symptoms) and MMSE total score. These three variables were evaluated for association with GM changes in all regions showing significant FESZ>HC GM reduction, a total of 25 regions. Thus, 3 clinical variables \times 25 regions = 75 chances for significance, and at a $p=.05$ level a chance association would predict 3.75 occurrences of significance. We found 17 significant occurrences of significance, a number that is greater than expected at a p level $<10^{-6}$ by the binomial theorem. Second, we must label the volume reduction in the cerebellum as questionable because of technical factors, since some images contained a few voxels with unusually high intensity in the cerebellum. Finally, we need to confirm the current VBM findings of the progressive GM volume reductions in the frontal and parietal regions using manually traced ROIs, studies that are now underway.

5. Conclusions

In conclusion, we believe the rich findings of longitudinal GM loss and associated clinical correlations in this VBM study are solidly based on the following integral and distinctive methodological features of this study: 1) use of the DARTEL algorithm and its improved accuracy in warping to templates; 2) creation of a more accurate subject alignment for detecting longitudinal GM changes by constructing individual Time1-Time2 templates before performing a group analysis; and 3) confirmation of this automated methodology by demonstrating the congruence with the findings of our previous ROI studies. ROI-VBM congruence was indicated by using SVC based on the ROI findings, and, like the ROI studies, finding progressive GM volume reductions, in the left STG and HG (Kasai et al., 2003a; Kasai et al., 2003b), and the bilateral anterior and posterior CG (Koo et al., 2008). To the best of our knowledge, this is the first study in which longitudinal manually traced ROI findings were used to validate VBM analyses using the same subjects and scanners. This longitudinal study also provides insight into the nature of the associations between brain morphology and clinical profile in patients with FESZ.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

VBM	voxel-based morphometry
DARTEL	Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra
SVC	small volume correction
ROI	region of interest
FDR	false discovery rate
FESZ	first-episode schizophrenia
HC	healthy control subjects
GM	gray matter
STG	superior temporal gyrus
HG	Heschl's gyrus
A/PCG	anterior/posterior cingulate gyrus
NCGM	neocortical gray matter
MMSE	Mini-Mental State Examination
BPRS	Brief Psychiatric Rating Scale

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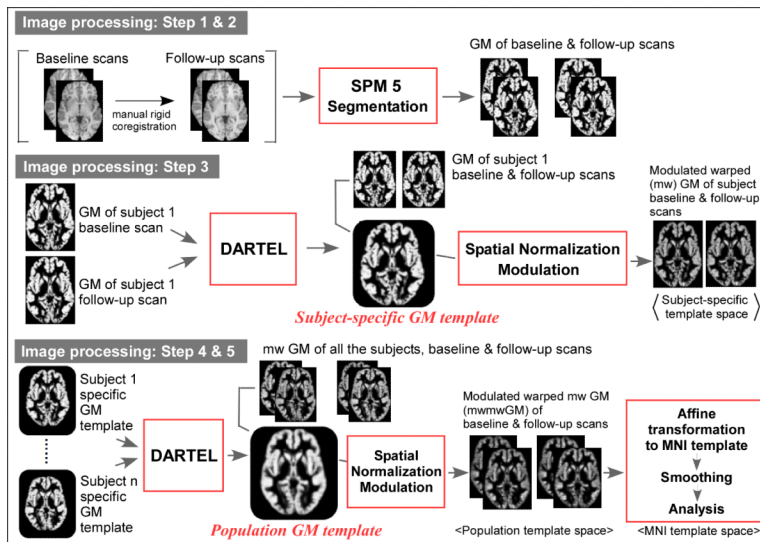


Figure 1.

Image processing of the longitudinal study using a Diffeomorphic Anatomical Registration

Through Exponentiated Lie algebra (DARTEL) tool in Statistical Parametric Mapping

(SPM) 5. Abbreviations: GM, gray matter; MNI, Montreal Neurological Institute

Step 1: Both baseline and follow-up scan T1-weighted images were realigned manually according to the AC-PC line and midsagittal plane. The baseline image was manually coregistered to the follow-up scan image without reslicing.

Step 2: All the T1-weighted images were segmented into probability maps of GM, white matter (WM) and cerebrospinal fluid by the unified segmentation approach in SPM5. The resulting GM and WM probability maps were automatically rigidly aligned (3 rotations and 3 translations) to MNI space and resampled into 1.5mm isotropic voxels.

Note: We did not describe WM maps in this figure because WM maps were only used to achieve better registration. Only GM maps were used for statistical analysis.

Step 3: To evaluate the longitudinal morphometric changes within each subject, a template was created for each subject using the information of both GM and WM maps. This template was obtained by combining the GM/WM maps of the baseline and follow-up scans into average GM/WM maps using an automated unbiased template building, non-linear registration program (DARTEL). The baseline and second scan GM/WM maps were then spatially normalized onto the corresponding *subject-specific* template non-linearly. The signal intensity of the normalized maps was modulated by the determinant of the Jacobian of the transformation to account for expansion and/or contraction of brain regions.

Step 4: A *population* template was created by simultaneously non-linearly registering all *subject-specific* GM/WM templates using DARTEL. The baseline and follow-up scan normalized maps of each subject were spatially non-linearly normalized to the population template, and then modulated.

Step 5: In order to bring the final analysis into standard MNI space, the *population* GM template was registered automatically to the MNI space through an affine transformation. All the individual GM maps residing in the *population* template space were then co-registered to MNI using the same affine transformation. Finally, these GM images were smoothed with an 8-mm FWHM Gaussian kernel. The information of the WM maps was not used in this step as it was only used to achieve better registration in steps 1-4, but not incorporated in the statistical analysis.

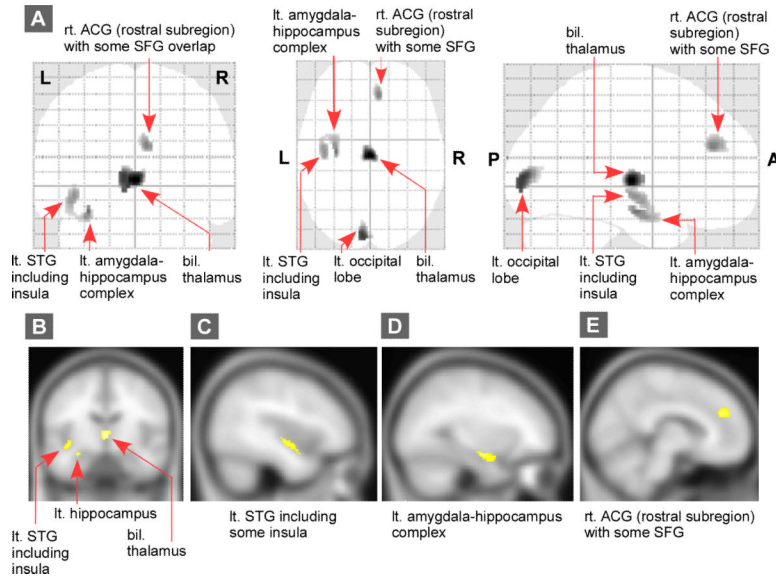


Figure 2.

Regions of reduced gray matter volume at baseline in the 33 patients with first-episode schizophrenia compared with the 36 healthy control subjects. Glass brain images include all the volume reduced regions (A). A coronal slice shows volume reductions of left superior temporal gyrus (STG) including some insula, hippocampus, and bilateral thalamus (B). Sagittal slices show volume reductions of the left STG including some insula (C), left amygdala-hippocampus complex (D), right anterior cingulate gyrus (ACG, rostral subregion) with some superior frontal gyrus (SFG) overlap (E). Uncorrected threshold of $p < .001$ with an extent threshold of 70 voxels is applied for graphical reporting (see supplemental Table S1 for specific coordinates). Abbreviations: L/lt, left; R/rt, right; A, anterior; P, posterior

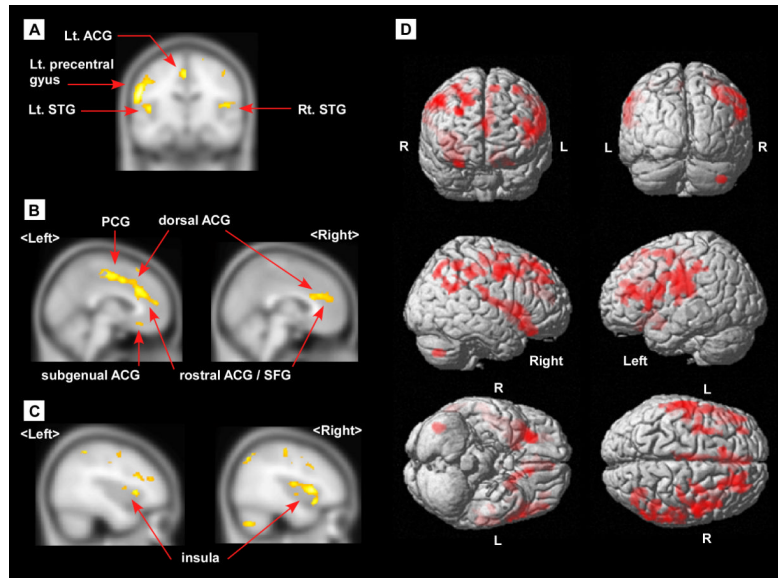


Figure 3.

Figure 3-A, B, C and D show progressive gray matter volume reductions greater in the 21 patients with first-episode schizophrenia compared with the 23 healthy control subjects over the 1.5 year follow-up interval. These regions include the bilateral superior temporal gyrus (STG), precentral gyrus, and left anterior cingulate gyrus (ACG) (A), left ACG (dorsal, rostral, and subgenual subregions), posterior cingulate gyrus (PCG), and superior frontal gyrus (SFG), and right ACG (dorsal and rostral) and SFG (B), left and right insula (C). Figure 3-D shows widespread gray matter volume reductions in the bilateral frontal, temporal and parietal regions (D) (see Table 3 for specific coordinates). Uncorrected threshold of $p < .001$ with an extent threshold of 70 voxels is applied for graphical reporting. Abbreviations: L/Lt, left; R/Rt, right; A, anterior; P, posterior

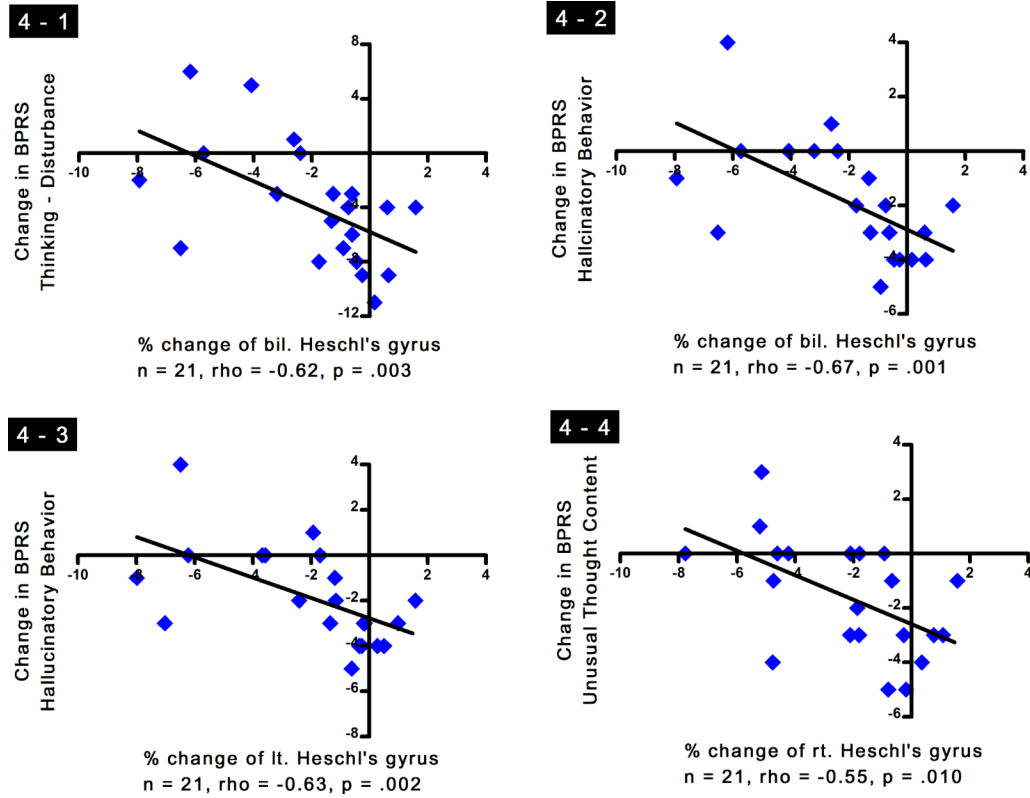
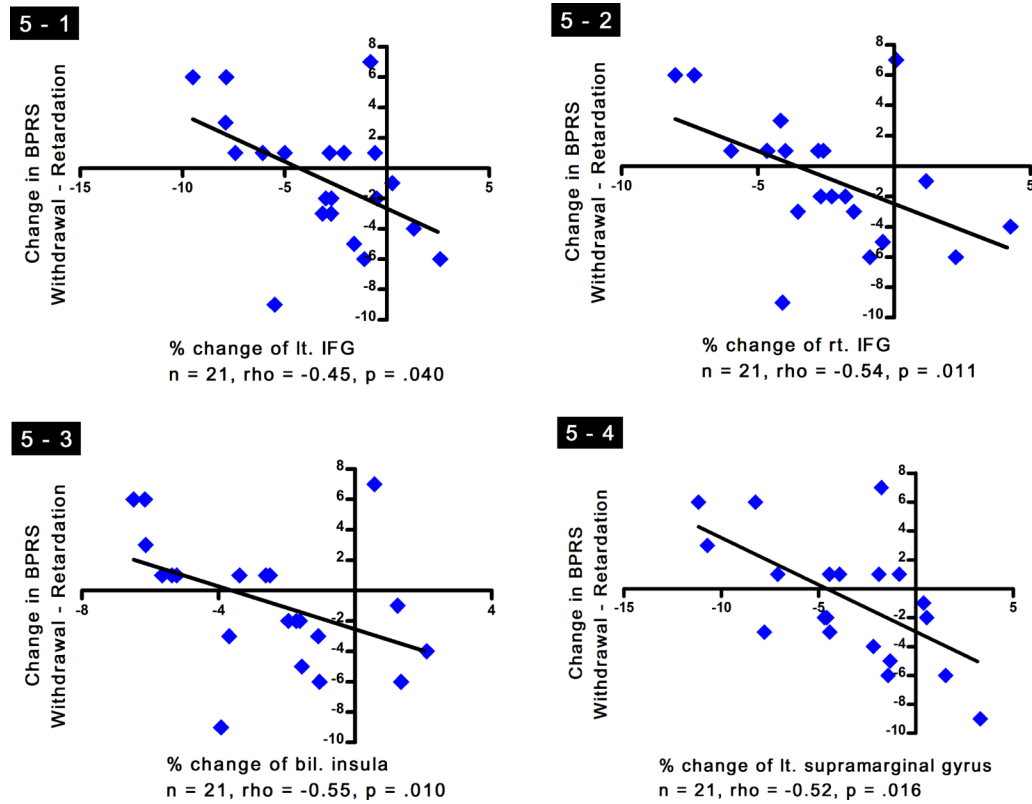


Figure 4.

Correlation between percentage change of regional gray matter (GM) volumes and absolute changes in thinking-disturbance (positive symptom), hallucinatory behavior, and unusual thought contents scores of Brief Psychiatric Rating Scale (BPRS). The lesser improvement of the thinking-disturbance (positive symptom) score was correlated with the greater longitudinal GM volume loss of the bilateral Heschl's gyrus (4-1). Lesser improvement of the hallucinatory behavior score was correlated with the greater longitudinal GM volume loss of the bilateral Heschl gyrus (4-2) and left Heschl's gyrus (4-3). Moreover, the lesser improvement of the unusual thought content score was correlated with the greater longitudinal GM volume loss of the right Heschl's gyrus (4-4). Abbreviations: lt, left; rt, right; bil, bilateral

**Figure 5.**

Correlation between percentage change of regional gray matter (GM) volumes and absolute changes in withdrawal-retardation (negative symptom) score of Brief Psychiatric Rating Scale (BPRS). Lesser improvement of the withdrawal-retardation (negative symptom) score was correlated with the greater longitudinal GM volume loss of the left and right inferior frontal gyrus (IFG, 5-1, -2), bilateral insula (5-3), and left supramarginal gyrus (5-4). Abbreviations: lt, left; rt, right; bil, bilateral

Table 1

Demographic and Clinical Characteristics of Cross-sectional Study Subjects

	<u>FESZ Group (n=33)</u>	<u>HC Group (n=36)</u>		
	Mean (SD)	Mean (SD)	df ^a	t-test or chi-square values ^b
Age, mean (SD) [range], years	22.5 (6.7) [18-45]	22.9 (3.8) [18-34]	67	1.27
Gender (male/female)	28 / 5	30 / 6	1	.03
Handedness ^c	0.80 (0.22)	0.78 (0.20)	66	.24
Socioeconomic Status ^d				
Subject's	3.4 (1.3)	2.3 (0.9)	67	4.17 <.001**
Parental	1.9 (0.9)	1.5 (0.7)	67	1.87
Years of education	13.5 (2.2)	15.0 (1.8)	65	2.98 .004**
WAIS-R information, scaled	11.7 (2.9)	13.4 (2.3)	60	1.64 .009**
WAIS-R Digit Span, scaled	10.2 (2.6)	11.3 (2.8)	60	1.59
Duration of illness, weeks	19.5 (20.3)	NA		
Antipsychotic medication dosage, CPZ equivalent	287.9 (181.0)	NA		
Medication use, No. of patients				
Neuroleptics, TYP/ATYP/Overlap/Non-neuroleptics ^e	7 / 19 / 6 / 1			
Li/VPA/Overlap/Non-mood stabilizer	2 / 5 / 0 / 26			
Duration of antipsychotic medication before scan, median [range], weeks	1.0 [0-20]	NA		
MMSE	28.1 (2.3)	28.9 (1.1)	63	1.65
BPRS	39.2 (11.0)	NA		
GAS	35.9 (8.1)	NA		

Abbreviations: FESZ, first-episode schizophrenia; HC, healthy control; WAIS-R, Wechsler Adult Intelligence Scale-Revised; CPZ, chlorpromazine; MMSE, Mini-Mental State Examination; BPRS, Brief Psychiatric Rating Scale; GAS, Global Assessment Scale; NA, data not applicable.

*p < .05

**p < .01.

^aThe degrees of freedom differ among variables owing to unavailability of data in some participants.

^bt tests were performed between the two groups for age, handedness, SES, parental SES, years of education, WAIS-R Information and Digit Span, scaled scores, and MMSE scores. A chi-square test was performed for sex ratio between the two groups.

^cHandedness was using the Edinburgh Handedness Inventory, where right handedness is positive.

Higher scores mean lower socioeconomic status, based on the Hollingshead two factor index of socioeconomic status.

^e One patient was antipsychotics-naïve at the baseline scan.

Table 2

Demographic and Clinical Characteristics of Longitudinal Study Subjects

	<u>FESZ Group (n=21)</u>		<u>HC Group (n=23)</u>		
	Mean (SD)		Mean (SD)		P
Age, mean (SD) [range], years					
Baseline Scan	25.0 (8.1) [18-45]		24.2 (3.9) [18-34]	42	.40
Second Scan	26.3 (7.8) [18-46]		25.5 (4.0) [19-36]	42	.66
InterScan Interval, months	17.8 (11.9)		16.4 (5.5)	42	.62
Gender (male/female)	18 / 3		19 / 4	1	.78
Handedness ^c	0.79 (0.24)		0.82 (0.22)	42	.13
Socioeconomic Status ^d					
Subject's	3.4 (1.4)		2.3 (1.0)	42	2.89
Parental	1.9 (0.7)		1.5 (0.7)	42	1.68
Years of education	13.4 (2.3)		15.4 (1.9)	42	3.10
WAIS-R information, scaled	12.0 (2.9)		13.5 (2.3)	38	1.88
WAIS-R Digit Span, scaled	10.0 (2.4)		11.5 (2.9)	38	1.87
MMSE					
Baseline Scan	27.7 (2.5)		28.9 (1.3)	40	2.02
Second Scan	28.0 (2.4)		29.4 (0.8)	41	2.50
BPRS total score					
Baseline Scan	41.8 (12.3)		NA		
Second Scan	28.0 (7.2)		NA		
BPRS thinking-disturbance factor (positive symptom)					
Baseline Scan	9.1 (3.7)		NA		
Second Scan	5.2 (2.1)		NA		
BPRS withdrawal-retardation factor (negative symptom)					
Baseline Scan	7.3 (2.8)		NA		
Second Scan	6.6 (3.5)		NA		
GAS					
Baseline Scan	35.9 (7.6)		NA		

	FESZ Group (n=21)		HC Group (n=23)		
	Mean (SD)		Mean (SD)		P
Second Scan	52.3 (12.9)		NA		
Antipsychotic medication dosage at baseline scan, CPZ equivalent	293.4 (181.2)		NA		
Medication use, No. of patients					
Neuroleptics, TYP/ATYP/Overlap/Non-neuroleptics ^e					
Baseline Scan	4 / 10 / 6 / 1		NA		
Second Scan	0 / 15 / 1 / 5		NA		
Mood stabilizer, Li/VPA/Overlap/Non-mood stabilizer					
Baseline Scan	1 / 2 / 0 / 18		NA		
Second Scan	4 / 4 / 0 / 13		NA		

Abbreviations: FESZ, first-episode schizophrenia; HC, healthy control; WAIS-R, Wechsler Adult Intelligence Scale-Revised; MMSE, Mini-Mental State Examination; BPRS, Brief Psychiatric Rating Scale; GAS, Global Assessment Scale; CPZ, chlorpromazine; TYP, typical antipsychotics; ATYP, atypical antipsychotics; Li, lithium carbonate; VPA, valproic acid; NA, data not applicable.

Note: some patients received more than one antipsychotic at a time.

* p < .05

** p < .01.

^aThe degrees of freedom differ among variables owing to unavailability of data in some participants.

^bt tests were performed among the two groups for age, interscan interval, handedness, SES, parental SES, years of education, WAIS-R Information and Digit Span, scaled scores, and MMSE scores. A chi-square test was performed for sex ratio between the two groups.

^cHandedness was using the Edinburgh Handedness Inventory, where right handedness is positive.

^dHigher scores mean lower socioeconomic status, based on the Hollingshead two factor index of socioeconomic status.

^eOne patient was antipsychotics-naïve at the baseline scan. In this naturalistic sample, 4 FESZs reported medication noncompliance for more than 3 months before the follow-up scan and 1 FESZ received no medication at the second scan. The antipsychotic-treated FESZ patients received olanzapine (50%), risperidone (19%), clozapine (13%), quetiapine (13%), aripiprazole (13%), and perphenazine (6%).

Longitudinal Study - Progressive Gray Matter Volume Reductions Greater in the 21 FESZ compared with the 23 HC over the 1.5 year follow-up interval

Table 3

Anatomical location	MNI coordinates			Number of voxels	T value	Uncorrected p	FDR-corrected p
	x	y	z				
left hemisphere							
STG including HG, and expanding to insula	-46	-14	8	96	4.43	<.001	.005**
SFG	-10	22	60	72	3.98	<.001	.006**
IFG	-46	28	18		5.16	<.001	.004**
postcentral gyrus	-58	-12	28	1943	6.19	<.001	.004**
supramarginal gyrus	-62	-24	24		5.38	<.001	.004**
precentral gyrus expanding to MFG	-34	-2	58	210	4.24	<.001	.005**
ACG expanding to PCC, right ACG, and bilateral SFG	-4	28	30	849	4.74	<.001	.004**
ACG	-8	24	-14		4.35	<.001	.005**
orbitofrontal cortex	-22	6	-18	311	3.95	<.001	.007**
right hemisphere							
insula	32	22	-14		5.27	<.001	.004**
IFG expanding to STG, HG, orbitofrontal cortex, and postcentral gyrus	40	16	4	1603	5.33	<.001	.004**
SFG	30	-2	60	141	4.16	<.001	.006**
SFG	24	36	48	511	4.66	<.001	.005**
MFG	48	22	42		4.70	<.001	.004**
precentral gyrus expanding to postcentral gyrus	50	-4	46	710	5.17	<.001	.005**
superior parietal gyrus	32	-68	52		5.12	<.001	.004**
angular gyrus expanding to supramarginal gyrus	58	-52	28	633	4.95	<.001	.004**
right cerebellum	38	-68	-42	108	4.14	<.001	.006**

Abbreviations: FESZ, first-episode schizophrenia; MNI, Montreal Neurological Institute; STG, superior temporal gyrus; HG, Heschl's gyrus; S/M/IFG, superior/middle/inferior frontal gyrus; A/PCG, anterior/posterior cingulate gyrus; FDR, False Discovery Rate

** FDR-corrected $p < .01$